AMENDMENTS TO THE SPECIFICATION

IN THE WRITTEN DESCRIPTION:

Please amend the paragraph starting at page 1, line 7 as follows.

The present invention relates to a method for forecasting and evaluating the ~~pharmacokinetics~~ pharmacokinetic parameter of an injection containing lipid A analog, a quality evaluating method of the injection for ensuring the injection to have constant ~~pharmacokinetics~~ pharmacokinetic parameter, and a preparation process of the injection.

Please amend the paragraph starting at page 5, line 14 as follows.

No report has however been published yet on the definite forecasting or evaluation of the ~~pharmacokinetics~~ pharmacokinetic parameter of a lipid A analog based on the correlation between the measuring and evaluating results of the aggregate condition of the medicament in a solution and the ~~pharmacokinetics~~ pharmacokinetic parameter. In addition, a report has been made neither on a process for producing an injection preparation wherein the ~~pharmacokinetics~~ pharmacokinetic parameter of a lipid A analog has been controlled nor a quality assurance method for ensuring the

injection preparation to have predetermined ~~pharmacokinetics~~ pharmacokinetic parameter, each from the viewpoints of the state of the aggregates of the medicament (lipid) in a ~~solution.~~

Please amend the paragraph starting at page 6, line 12 as follows.

The injection preparation thus prepared is however accompanied with the problem that when it is administered to a rat or beagle, the blood level varies largely from one lot to another of a raw material medicament or the preparation. This owes to that the existing state of a lipid A analog in a solution, that is, the aggregate structure in the form of endoplasmic reticulum ~~with~~ of lipid biomolecular membrane or micelle having a diameter not greater than 30 nm differs with the lot of the raw material medicament or injection preparation. There is accordingly a strong demand for the development of a practically usable injection of a lipid A analog, that is, an injection having uniform ~~pharmacokinetics~~ pharmacokinetic parameter, which is typified by the blood level, without being influenced by the difference among the lots of a raw material medicament or injection preparation; and a forecasting and evaluating method of the ~~pharmacokinetics~~ pharmacokinetic parameter of the injection.

Please amend the paragraph starting at page 7, line 5 as follows.

With the foregoing in view, the present inventors have carried out an extensive investigation to search for an injection containing a lipid A analog, having high transparency and having good stability and in addition, exhibiting constant uniform ~~pharmacokinetics~~ pharmacokinetic parameter free from the influence of variations of a raw material medicament or injection preparation among lots; and forecasting and evaluating method of the ~~pharmacokinetics~~ pharmacokinetic parameter of the injection. As a result, it has been found that the object can be attained by the below-described constitutions, leading to the completion of the present invention.

Please amend the paragraph starting at page 7, line 16 as follows.

In one aspect of the present invention, there is provided a forecasting method of the ~~pharmacokinetics~~ pharmacokinetic parameter of a lipid A analog in an injection preparation containing the lipid A analog or pharmacologically acceptable salt thereof, which comprises measuring membrane fluidity and/or circular dichroism in a solution.

Please amend the paragraph starting at page 7, line 22 as follows.

In another aspect of the invention, there is also provided an evaluation method of the ~~pharmacokinetics~~ pharmacokinetic parameter of a lipid A analog in an injection preparation containing the lipid A analog or a pharmacologically acceptable salt thereof, which comprises measuring membrane fluidity and/or circular dichroism in a solution.

Please amend the paragraph starting at page 8, line 2 as follows.

In a further aspect of the present invention, there is also provided a quality assurance method of an injection preparation containing a lipid A analog or a pharmacologically acceptable salt thereof, which comprises measuring and evaluating membrane fluidity and/or circular dichroism in a solution, and ensuring the lipid A analog to exhibit constant ~~pharmacokinetics~~ pharmacokinetic parameter *in vivo*.

Please amend the paragraph starting at page 8, line 15 as follows.

The forecasting method of the ~~pharmacokinetics~~ pharmacokinetic parameter according to the present invention can be employed for the evaluation of an injection preparation,

quality evaluation for obtaining an injection preparation exhibiting constant ~~pharmacokinetics~~ pharmacokinetic parameter or preparation of the injection preparation.

Please amend the paragraph starting at page 8, line 21 as follows.

According to the present invention, an injection preparation which is transparent and is ensured to have stable ~~pharmacokinetics~~ pharmacokinetic parameter is available using a lipid A analog or a pharmacologically acceptable salt (which will hereinafter be called "lipid A analog", collectively). This is an object of the present invention. In addition, the present invention provides a forecasting and evaluating method of the ~~pharmacokinetics~~ pharmacokinetic parameter of an injection preparation containing the lipid A analog by measuring the membrane fluidity and/or circular dichroism; and also provides a quality evaluating method of an injection preparation containing a lipid A analog, which ensures the lipid A analog to exhibit constant ~~pharmacokinetics~~ pharmacokinetic parameter. This is another object of the present invention.

Please amend the paragraph starting at page 17, line 12 as follows.

No example of evaluating physicochemical properties of the

membrane of a preparation containing a lipid A analog has so far been proposed from the viewpoint of ~~pharmacokinetics~~ pharmacokinetic control.

Please amend the paragraph starting at page 17, line 16 as follows.

The present invention has revealed that in a step for preparing an injection preparation which contains aggregates having a diameter not greater than 30 nm by dissolving a lipid A analog in an alkaline solution and then adding a buffer to the resulting solution, a ~~pharmacokinetics~~ pharmacokinetic profile of the injection containing a lipid-A analog can be controlled by measuring and evaluating its membrane fluidity and circular dichroism.

Please amend the paragraph starting at page 17, line 24 as follows.

The membrane fluidity of the particles in an injection preparation using DPH has a close correlation with a blood level profile. Upon administration of the injection preparation, a preparation having larger membrane fluidity (a preparation having softer membrane) disappears more slowly from the blood (larger AUC), while a preparation having smaller membrane fluidity (a preparation having harder membrane) disappears more

rapidly from the blood (smaller AUC). This is because after administration of a lipid-A-containing injection, membrane fluidity affects the ~~pharmacokinetics~~ pharmacokinetic parameter of the medicament. The larger the membrane fluidity, the lipid A does not tend to be trapped easily by a scavenger receptor and is therefore not taken in a phagocyte such as liver cells readily, retarding the disappearance of lipid A from the circulating blood. The present invention has also revealed that there is a correlation between the CD spectrum change and variations in the ~~pharmacokinetics~~ pharmacokinetic parameter.

Please amend the paragraph starting at page 18, line 16 as follows.

The present invention makes it possible to prepare an injection preparation containing a lipid-A analog ensured to have good ~~pharmacokinetics~~ pharmacokinetic parameter by utilizing, for the measurement of membrane fluidity and/or circular dichroism, the fact that membrane fluidity and/or circular dichroism in the solution of the injection differs definitely between a formulation of a low disappearing rate and a formulation of a high disappearing rate from the blood. The present invention also makes it possible to provide a forecasting or evaluating method of the ~~pharmacokinetics~~ pharmacokinetic parameter of an injection preparation containing

a lipid-A analog by measuring membrane fluidity and/or circular dichroism; and a quality evaluating method for ensuring the injection to have constant ~~pharmacokinetics~~ pharmacokinetic parameter.

B13

Please amend the paragraph starting at page 19, line 4 as follows.

In the present invention, a highly-transparent injection preparation which has controlled ~~pharmacokinetics~~ pharmacokinetic parameter and contains aggregates having a diameter not greater than 30 nm can be prepared by dissolving a lipid A analog in an aqueous alkaline solution, adding thereto a buffer and measuring membrane fluidity and/or circular dichroism in the resulting solution. In the present invention, the membrane fluidity of an injection preparation is measured by the fluorescence probe method. This is a method for evaluating membrane fluidity of a bimolecular membrane structure of a phospholipid. Described specifically, the state of the membrane in the vicinity of a fluorescent substance is observed by mixing a fluorescence probe in the membrane of a lipid and measuring the polarity of fluorescence emitted upon exposure to polarized incident light. In the present invention, any one or more than one parameter selected from fluorescence polarity (P: ranging from 0 to 0.5), fluorescence anisotropy (r: ranging from 0 to

B14

0.4) and order parameter (S: ranging from 0 to 1.0) may be used for evaluation of membrane fluidity. As a fluorescence probe to be used, any one capable of emitting stable fluorescence can be employed. Examples include diphenylhexatriene (DPH), carboxyfluorescein, calcein, Nile Red, pyrene and perylene.

Please amend the paragraph starting at page 20, line 8 as follows.

The circular dichroism spectroscopy (CD spectroscopy) of an injection preparation employed in the present invention is useful as a method for controlling or forecasting the ~~pharmacokinetics~~ pharmacokinetic parameter of a lipid A analog. It is preferred to evaluate the CD spectrum of each of a number of injection preparations and select the wavelength at which a large difference in CD intensity can be recognized. In the CD spectroscopy of the lipid A analog represented by the formula (III) or (IV), wavelength measured is usually 260 to 320 mm, preferably 270 to 310 mm, more preferably 280 to 300 mm.

Please amend the paragraph starting at page 23, line 3 as follows.

One example of each of the measuring methods of membrane fluidity and circular dichroism (CD spectroscopy) of an injection preparation and the evaluation method of the

~~pharmacokinetics~~ pharmacokinetic parameter in a rat administered with the preparation will next be described.

Please amend the paragraph starting at page 24, line 20 as follows.

Evaluation method of ~~pharmacokinetics~~ pharmacokinetic parameter in a rat administered with an injection preparation

Please amend the paragraph starting at page 24, line 22 as follows.

The ~~pharmacokinetics~~ pharmacokinetic parameter profile of the preparation is judged using as a parameter an area under the curve available from the time-dependent curve of the blood level of medicament (this area will hereinafter be abbreviated as "AUC").

Please amend the paragraph starting at page 25, line 19 as follows.

According to the present invention, it is possible to provide an injection preparation containing a lipid A analog as that having high transparency and stably controlled ~~pharmacokinetics~~ pharmacokinetic parameter. Also a forecasting method and evaluating method of the ~~pharmacokinetics~~ pharmacokinetic parameter of an injection preparation and a

quality assurance method of an injection preparation containing a lipid A analog, which ensures the preparation to have constant ~~pharmacokinetics~~ pharmacokinetic parameter can be provided. The following are the advantage examples of the present invention.

Please amend the paragraph starting at page ~~27,~~ line 14 as follows.

Evaluation of the membrane fluidity and circular dichroism of the preparations different in the profile of ~~pharmacokinetics~~ pharmacokinetic parameter (AUC), according to the present invention

Please amend the paragraph starting at page 29, line 11 as follows.

In CD intensity as measured by circular dichroism (CD spectroscopy), as shown in Fig. 2, a clear difference was recognized between them at wavelength of 280 nm. The above-described findings show clearly that evaluation of membrane fluidity and/or circular dichroism is useful for evaluating a change in the ~~pharmacokinetics~~ pharmacokinetic parameter of the lipid-A-analog-containing injection preparation according to the present invention.

Please amend the paragraph starting at page 30, line 15 as follows.

Concerning an injection preparation containing the lipid A analog represented by the formula (III), ten lots were prepared and evaluated. As a result, a negative correlation was recognized between the AUC of a rat and order parameter (S) as shown in Fig. 6 and its correlation coefficient was 0.873. Concerning the correlation between AUC of a rat and the average particle diameter of the injection preparation, AUC showed large variations at an average particle diameter ranging from 10 to 20 mm and therefore, no definite correlation was recognized (Fig. 7) between them. Accordingly, evaluation using an average particle size was insufficient for forecasting and evaluation of ~~pharmacokinetics~~ pharmacokinetic parameter.

Please amend the paragraph starting at page 31, line 2 as follows.

Thus, a good correlation of a ~~pharmacokinetics~~ pharmacokinetic parameter profile (AUC) with evaluation of membrane fluidity and/or circular dichroism of the injection preparation according to the present invention has been proved. It is evident that use of membrane fluidity (order parameter (S)) and circular dichroism (CD) is fully effective for forecasting and evaluating the ~~pharmacokinetics~~ pharmacokinetic

parameter of an injection preparation containing a lipid A analog. In addition, it becomes possible to prepare an injection preparation having ensured ~~pharmacokinetics~~ pharmacokinetic parameter by evaluating the state of aggregates in the solution based on membrane fluidity and/or circular dichroism. In other words, AUC can be presumed by measuring the order parameter (S) and/or circular dichroism (CD) of an injection preparation containing lipid A analog. Accordingly, the present invention can therefore be used industrially and useful.

Please amend the paragraph starting at page 41, line 1 as follows.

In a glass beaker, 100 mg of the lipid A analog represented by the formula (III) was weighed, followed by the addition of 50 mL of 0.01M NaOH. The resulting mixture was stirred by a stirrer at room temperature (about 25°C). To the reaction mixture was added 600 mL of a lactose·phosphate buffer (an aqueous solution obtained by dissolving in 600 mL of distilled water for injection 100 g of lactose monohydrate, 0.45 g of $Na_2HPO_4 \cdot 7H_2O$ and 0.35 g of $NaH_2PO_4 \cdot H_2O$). After stirring by a stirrer, a proper amount of distilled water for injection was added to adjust the total amount to 1 L. The medicament solution thus obtained was filtered through a 0.22 μm filter.

Then, 5.3 ml portions of the filtrate were pipetted into vials, followed by freeze-drying. To each of the vials containing the freeze-dried injection preparation, 5 ml of distilled water for injection was added to thaw the preparation. The evaluation results of the ~~pharmacokinetics~~ pharmacokinetic parameter (AUC) of a rat, particle size, and membrane fluidity (order parameter (S), fluorescence anisotropy r, fluorescence polarity P) are shown in Table 2.

Please amend the paragraph starting at page 41, line 22 as follows.

In a glass beaker, 100 mg of the lipid A analog represented by the formula (III) was weighed, followed by the addition of 150 mL of 0.01M NaOH. The resulting mixture was stirred by a stirrer at room temperature. After visual conformation of the disappearance of a gel of the lipid analog A represented by the formula (III), the glass beaker was subjected to ultrasonic exposure at a temperature maintained at 10°C or less in a bath type sonicator. To the reaction mixture was added 600 mL of a lactose·phosphate buffer (an aqueous solution obtained by dissolving in 600 mL of distilled water for injection 100 g of lactose monohydrate, 0.45 g of $Na_2HPO_4 \cdot 7H_2O$ and 0.35 g of $NaH_2PO_4 \cdot H_2O$). After stirring by a stirrer, a proper amount of distilled water for injection was added to adjust the total

amount to 1 L. The medicament solution thus obtained was filtered through a 0.22 μm filter. Then, 5.3 ml portions of the filtrate were pipetted into vials, followed by freeze-drying. To each of the vials containing the freeze-dried injection preparation, 5 mL of distilled water for injection was added to thaw the preparation. The evaluation results of the ~~pharmacokinetics~~ pharmacokinetic parameter (AUC) of a rat, particle diameter, and membrane fluidity (order parameter (S), fluorescence anisotropy r, fluorescence polarity P) are shown in Table 2.

Please amend the paragraph starting at page 45, line 2 as follows.

In a glass beaker, 100 mg of the lipid A analog represented by the formula (III) was weighed. After addition of 50 mL of 0.003M NaOH, the resulting mixtures were stirred at 50 ± 5 °C for 3, 8, 15, 30 and 90 minutes, respectively. To each of the reaction mixtures, 600 mL of a lactose·phosphate buffer (an aqueous solution obtained by dissolving in 600 mL of distilled water for injection 100 g of lactose monohydrate, 0.45 g of $Na_2HPO_4 \cdot 7H_2O$ and 0.35 g of $NaH_2PO_4 \cdot H_2O$) was added. After stirring by a stirrer, a proper amount of distilled water for injection was added to adjust the total amount to 1 L. The medicament solution thus obtained was filtered through a 0.22 μm filter.

Then, 5.3 ml portions of the filtrate were pipetted into vials, followed by freeze-drying. To each of the vials containing freeze-dried injection preparation, 5 ml of distilled water for injection was added to thaw the preparation. The evaluation results of the ~~pharmacokinetics~~ pharmacokinetic parameter (AUC) of a rat, particle diameter, membrane fluidity (order parameter (S)) and surface charge are shown in Table 3. Since stirring was conducted in a sodium hydroxide solution at a temperature (50 ± 5°C) not less than the phase transfer temperature (about 30°C) of the lipid A analog represented by the formula (III), AUC depended on the stirring time and the longer the stirring time, the greater AUC. It has also been recognized that the greater AUC, the smaller order parameter (S). Between AUC and the particle diameter or surface charge, no definite correlation was recognized.

Please amend the paragraph starting at page 16, line 2 as follows.

In a glass beaker, 100 mg of the lipid A analog represented by the formula (III) was weighed, followed by the addition of 50mL of 0.003M NaOH. The resulting mixture was stirred by a stirrer at 50±5°C for 30 minutes. To the reaction mixture was added 600 mL of a lactose·phosphate (an aqueous solution

obtained by dissolving in 600 mL of distilled water for injection 100 g of lactose monohydrate, 0.45 g of $Na_2HPO_4 \cdot 7H_2O$ and 0.35 g of $NaH_2PO_4 \cdot H_2O$). After stirring by a stirrer, a proper amount of distilled water for injection was added to adjust the total amount to 1 L. The resulting medicament solution was filtered through a 0.22μm filter. Each of 5.3 ml portions of the filtrate was pipetted into a vial and then freeze-dried. The initial stage product and products tested for stability under the conditions of 40°C and 75% RH (freeze-dried injection preparations stored for 1, 2 and 3 months) were thawed with 5 ml of distilled water for injection with the replicate of 3 and their membrane fluidity (order parameter (S)) was evaluated. The results are shown in Table 4. Variations in the order parameter (S) were small upon measurement, indicating that the reproducibility was sufficient. In addition, it was revealed that the injection preparations, whether they were initial stage products or stored for 3 months, were stable free from variations in order parameter (S) and ~~pharmacokinetics~~ pharmacokinetic parameter.